Biomedicine & Pharmacotherapy 112 (2019) 108638

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Vindoline effectively ameliorated diabetes-induced hepatotoxicity by docking oxidative stress, inflammation and hypertriglyceridemia in type 2 diabetes-induced male Wistar rats

Mediline Goboza[a], Yapo G. Aboua[b], Novel Chegou[c], Oluwafemi O. Oguntibeju[a,*]

[a] Department of Biomedical Sciences, Phytomedicine and Phytochemistry Research Group, Oxidative Stress Research Centre, Faculty of Health & Wellness Sciences, Cape Peninsula University of Technology, P.O. Box 1906, Bellville, 7535, South Africa
[b] Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Private Bag, 13388, Windhoek, Namibia
[c] Department of Biomedical Sciences, Division of Molecular Biology and Human Genetics, DST/NRF Centre of Excellence for Biomedical Tuberculosis Research and SAMRC Centre for Tuberculosis Research, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

ARTICLE INFO

Keywords:
Type 2 diabetes mellitus
Oxidative stress
Inflammatory cytokines
Hepatic injury
Vindoline

ABSTRACT

Vindoline, an indole alkaloid present in the leaves of *Catharanthus roseus* plant, has been recently reported to have insulotropic effects. This present study evaluated the possible hepatoprotective effects of vindoline in a type 2 diabetes mellitus rat model. Diabetes mellitus was induced by exposing rats to 10% fructose water for two weeks followed by a single intraperitoneal injection of 40 mg/kg body weight of streptozotocin (STZ). Rats were randomly divided into six groups (n = 8) and treated daily for 6 weeks with the vehicle via oral gavage, vindoline (20 mg/kg) or glibenclamide (5 mg/kg). Weekly fasting blood glucose (FBG) levels and body weight were measured and recorded. Administration of vindoline significantly ($p < 0.05$) reduced FBG by 15% when compared to the diabetic controls. Vindoline significantly ($p < 0.05$) decreased diabetes-induced hepatic injury shown by decreased levels of serum alanine transferase (ALT) (-42%), aspartate aminotransferase (AST) (-42%) and alkaline phosphatase (-62%) compared to the diabetic controls. The oxygen radical absorbance capacity and the activities of superoxide dismutase (SOD) and catalase (CAT) were also improved following treatment with vindoline. The results also showed decreased levels of pro-inflammatory cytokines such as TNF-α by (-41%) and IL-6 (-28%) which may have also contributed to the reduction of serum triglycerides (-65%) in the diabetic group treated with vindoline. Histopathological findings showed improvement of both the hepatic and pancreatic tissues following vindoline treatment. Overall, these findings suggest that vindoline may protect the diabetic hepatic tissue from injury via antioxidant, anti-inflammatory and anti-hypertriglyceredemia mechanisms thereby retarding the development of diabetic complications.

1. Introduction

Diabetes mellitus (DM) has recently been labelled as one of the chronic diseases that has become a global health threat [1]. According to the World Health Organisation (WHO) 2016 report [2], an estimate of 422 million people globally were diagnosed with DM in 2014 while a 108 million had diabetes in 1980. DM is also one of the four most common non-communicable-diseases and currently the seventh leading cause of morbidity and mortality globally [3]. This high mortality rate is associated with complications such as diabetic liver disease (DLD) which progressively develop in these patients. Liver cirrhosis, steatohepatitis, non-alcoholic fatty liver disease (NAFLD) and hepatic carcinomas are examples of different spectrums of DLDs that are observed in T2DM patients with persistent hyperglycaemia [4,5]. DLDs primarily develop as a consequence of hyperglycaemia, hypertriglyceridemia and insulin resistance. In the absence of sufficient cytoprotective molecules, these cofactors induce inflammation, oxidative damage and finally necrosis or apoptosis [5,6]. In diabetes, chronic hyperglycaemia provides an environment that facilitates free radical formation, concomitantly depleting the endogenous antioxidant reserves ultimately leading to oxidative tissue damage [7,8]. Additionally, increased levels of free radicals activate pro-inflammatory cytokines and the transcription of pro-apoptotic genes thus mediating chronic inflammatory responses and hepatocyte death respectively [9]. Abnormal high glucose levels have the ability to induce apoptosis by activating Bax–caspase proteases that alter mitochondrial function leading to apoptosis [10].

* Corresponding author.
E-mail address: oguntibejuo@cput.ac.za (O.O. Oguntibeju).

https://doi.org/10.1016/j.biopha.2019.108638
Received 19 October 2018; Received in revised form 9 January 2019; Accepted 28 January 2019

Therapeutic strategies which can efficiently prevent or reverse the adverse effects of prolonged oxidative stress and inflammation in DM may be highly beneficial in preventing diabetic complications such as DLDs.

From past decades, concerted efforts were made in attempts to manage diabetes. These efforts evoked the discovery of different orthodox drugs currently used to achieve better glycaemic control and thus prevention of DM complications. A major drawback of oral antidiabetic agents is their failure to effectively cure diabetes; moreover, they have been associated with increased side effects [11]. In this regard, medicinal plants appear as promising therapeutic avenues motivated by the presence of phytoconstituents that have numerous health benefits and may act as precursors in drug formulations [12–14].

Catharanthus roseus or *Vinca rosea* is a medicinal plant that has its origin in Madagascar hence the other name Madagascar periwinkle [15–18]. Traditionally, it was used to treat diseases like cancer, malaria, diabetes, insomnia and high blood pressure [18–21]. Different scientific studies reported the anti-hyperglycaemic nature of different extracts of *C. roseus* attributed to the presence of indole alkaloids, flavonoids, beta sitosterol, quercetin, catharanine [17,22–26]. Vindoline is an indole alkaloid extracted from *C.roseus* which was recently been reported to possess protein-tyrosine phosphatase 1B (PTP-1B) inhibitory effects and therefore may serve as an "insulin sensitizer" in the management of T2DM [18,21]. To the best of our knowledge there is limited knowledge/information on the effects of vindoline in T2DM and associated complications. The present study was undertaken to evaluate the effect/s of vindoline against hyperglycaemia-induced oxidative tissue damage in the liver of T2DM- induced male Wistar rats.

2. Materials and methods

2.1. Animal care

Six weeks old male Wistar Rats were purchased from Charles River (Margate United Kingdom). Animals were housed at the South African Medical Research Council (PUDAC) according to the national standards and policies set out in the South African National Standard for the Care and Use of Animals for Scientific Purposes (South African Bureau of Standards SANS (10386:2008). Six rats per cage were housed under controlled standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity, 12:12 h cycles of light to darkness. All the rats had free access to water and standard laboratory diet called Standard Rat Chow (SRC).

2.2. Ethical approval

This study was granted approval from the Ethics Committee for Research on Animals of the South African Medical Research Council (REF-01/17) and from the Faculty of Health and Wellness Research Ethics Committee of Cape Peninsula University of Technology, South Africa (CPUT/HW-REC 2016/A4).

2.3. Plant-derived chemical and standard drug

Vindoline was purchased from the manufacturer: Best of Chemicals (BOC) Sciences, USA). Specific guidelines, storage and preparation methods were followed as per suppliers' instructions. The standard drug glibenclamide was purchased from a local pharmacy and used in the experiment.

2.4. Induction of T2DM

T2DM was induced in rats by having them drink 10% fructose solution *ad libitum* for 14 days. Non-diabetic rats only drank normal tap water ad libitum. On day 15, rats received a single intraperitoneal injection of low dose of streptozotocin (STZ, 40 mg/kg body weight (b.w)) in freshly prepared 0.1 M citrate buffer (pH 4.5) after an overnight fast. Diabetes was confirmed 72 h post STZ administration, rats with 4 h fasting blood glucose level; between 15 mmol/ and 40 mmol/l were considered diabetic. Daily treatment (for 6 weeks) through oral gavage with respective compounds (vindoline/ glibenclamide) commenced after 5 days of STZ administration to ensure stable hyperglycaemia.

2.5. Experimental design

Forty-eight (48), 6 weeks old male Wistar rats (190–230 g) were randomly divided into six (6) groups with a minimum of eight rats each (n = 8). Rats in all the groups were fed *ad libitum* with standard rat chow (SRC) and tap water.

Group **N**: normal control administered with vehicle only;

Group **NV**: normal treated group administered with vindoline (20 mg/kg b.w);

Group **NG:** normal treated group treated with glibenclamide (5 mg/kg b.w).

Group **DC:** diabetic control group administered with vehicle;

Group **DV:** diabetic treated with 20 mg/kg b.w of vindoline;

Group **DM:** diabetic treated with 5 mg/kg b.w of glibenclamide;

Fasting blood glucose levels and weights were measured once a week using a glucometer and a balance respectively while blood samples were collected via the tail prick. After the 8 week study period, the rats were fasted overnight in preparation for sacrifice and sample collection. Final blood glucose levels and body weights were recorded prior to termination of experiment. Rats were anaesthetised and euthanized using isoflurane gas at 2% with 1% oxygen during laparotomy.

2.6. Serum preparation

Blood samples were collected from the abdominal vena cava into 10 ml serum separator vacutainer tubes (yellow top tubes). Tubes were allowed to stand at room temperature for 30 min prior to centrifugation. The samples were centrifuged at 3500 rpm for 15 min. Once centrifuged, the serum and plasma were aliquoted into cryo-tubes, snap-frozen in liquid nitrogen and stored at −80 °C.

2.7. Organ preparation

Liver samples were collected, quickly washed in phosphate buffered saline (PBS) to remove blood and then weighed. Some portions to be used for antioxidant analysis were snap frozen in liquid nitrogen and stored at −80 °C for future analysis. Remaining portions were preserved in 10% (v/v) neutral buffered formalin and embedded in paraffin wax for histological analysis. Later on, the snap frozen samples were homogenised in ice-cold respective buffers for different endogenous antioxidant activity determination. Homogenates were centrifuged for 15 min at 15,000 rpm at 4 °C, aliquoted and stored at −80 °C until analysis.

2.8. Determination of the relative liver weights

The relative liver weights were calculated using the following formula

Relative liver weight = (liver weight ÷ total body weight) 100g

2.9. Liver function enzymes activity

The activities of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured using ABX PENTRA 400 automated chemistry analyser machine.

2.10. Measurement of hepatic antioxidant marker enzymes and reduced GSH level

Catalase (CAT) activity was determined spectrophotometrically by assessing the exponential disappearance of H_2O_2 at 240 nm. The assay was carried out according to the method of Aebi [27]. The activity of superoxide dismutase (SOD) was measured following the procedure described by Crosti et al [28]. The level of reduced glutathione (GSH) was determined by the method of Jollow et al [29] and measured spectrophotometrically at 412 nm.

2.11. Determination of lipid peroxidation

Lipid peroxidation (LPO) in the liver homogenates was assessed by measuring the amount of malondialdehyde (MDA) products that were reacted with the thiobarbituric acid. The coloured complex formed when MDA reacted with thiobarbituric acid (TBA) was measured at 532 nm according to the method used by Tug et al [30].

2.12. Hepatic antioxidant analysis

Oxygen radical absorbance capacity (ORAC) is an assay that measures the ability of antioxidants in a particular sample to scavenge radicals [31]. The principle of the assay is based on the scavenging and inhibition capacity of lipophilic antioxidants in the presence of a cyclodextrin water-based enhancer upon the free radical damages. The free radical damage is evaluated by the loss of fluorescence of a fluorescent probe over time; therefore the decrease of fluorescence signifies the extent of free radical damage and as well as a direct proportional relationship with the free radical concentration [31,32].

2.13. Measurement of triglycerides

The concentration of triglycerides in the serum was determined using the ABX PENTRA 400 chemistry analyser. The kit was purchased from Scientific Group Company (South Africa).

2.14. Inflammatory cytokines

The amount of cytokines: interleukin-6 (IL-6), IL-10, IL-1b and tumor necrosis factor (TNF)-α in the homogenates were evaluated using the Bio-plex® platform (Bio-rad Laboratories, Hercules, CA (USA). The MILLIPLEX® MAP rat cytokine magnetic bead-based Luminex kit was purchased from Merck Millipore, Billerica, MA (USA).

2.15. Histopathological studies

Washed liver samples were fixed in 10% formalin, processed using routine histology techniques. The liver samples were then embedded in paraffin and were cut into sections of 5 µm thickness using the Leica RM2125 microtome (Leica Microsystems, Inc., Buffalo Grove, United States of America). The sections were deparaffinised and stained using haematoxylin and eosin (H & E) stain

2.16. Immunohistochemistry

Anti-Bcl2, anti-caspase 3 and anti-caspase 9 antibodies were obtained from Abcam. Formalin-fixed, paraffin-embedded liver sections were first pre-treated with heat-induced epitope retrieval (HIER) for 20 min at 98 °C so as to expose antigen sites. Respective antibodies at a dilution of 1:100 were added to the slides. Immuno labelling staining was performed using the Leica Bond autostainer (Leica Biosystems, SA). Slides were incubated with peroxidase block for 5 min using the Bond Polymer Refine Detection Kit. The sections were then incubated for 30 min in respective primary antibodies. Following incubation, post primary block incubation using post primary antibody was performed at room temperature for 30 min. Incubation of slides with 3,3′ Diaminobenzidine (DAB) chromogen solution and DAB substrate buffer polymer was done to facilitate the production of a brown end-product. Finally, hematoxylin was used to counterstain the nuclei during a 5 min incubation step. Liver tissue sections were dehydrated by moving slides in a series of graded alcohols. Slides were finally mounted using dibutyl phthalate xylene (DPX). Slides were viewed and images captured using the EVOS XL Cell imaging microscope. Positive intensities were analysed and quantified using ImageJ Immuno Profiler software (version 10.2 image analysis).

2.17. Statistical analysis

Results were analysed using GRAPH PAD Prism software package, Version 5.0. Data were expressed as mean ± standard error mean (SEM). The comparisons within groups were determined by using the one way analysis of variance (ANOVA) and Bonferonni's multiple test comparison. The values were considered to be statistically significant when the p value was < 0.05.

3. Results

3.1. Effect of vindoline or glibenclamide on body weight, liver weight and blood glucose levels

The effect of vindoline or glibenclamide administration on blood glucose, serum insulin, and body and liver weights in T2DM-induced and normal rats is represented in Table1 below. Treating diabetic rats with vindoline resulted in significant decrease in blood glucose level (15% decrease) in comparison with the diabetic control (DC) ($p < 0.05$) whereas glibenclamide did not show significant alterations in glucose levels when its effect on glucose levels was compared to both the diabetic controls as well as the diabetic group that received vindoline ($p > 0.05$). In normal rats, vindoline and glibenclamide caused no major changes in the glucose levels when compared to the normal controls. The insulin level in diabetic rats treated with vindoline (DV)

Table 1
Effect of vindoline or glibenclamide administration on glucose, serum isulin, body and liver weights in T2DM-induced and normal rats.

Parameters	NC	NV	NG	DC	DV	DG
FG (mmo/L	10.62 ± 0.34^{b}	10.04 ± 0.31^{b}	10.18 ± 0.29^{b}	31.94 ± 0.54^{a}	27.15 ± 1.47^{ab}	29.23 ± 1.33^{a}
Insulin µlU/ml	11.67 ± 1.03	7.43 ± 0.37^{a}	9.85 ± 0.92^{b}	5.13 ± 0.46^{a}	9.56 ± 1.32^{b}	8.47 ± 1.09
FBW(g)	293.1 ± 23.34^{b}	338.4 ± 37.05^{ab}	310.0 ± 29.45^{b}	243.1 ± 33.6	241.0 ± 26.64^{a}	255.8 ± 15.80
WC (%)	94.56	83.0^{b}	79.51^{b}	31.21^{a}	46.06^{a}	52.339^{a}
LW (g)	9.71 ± 1.18	11.31 ± 1.67	9.46 ± 1.07	11.97 ± 1.04	11.04 ± 1.54	12.02 ± 1.02
RLW (g)	3.32 ± 0.38^{b}	3.34 ± 0.27^{b}	3.05 ± 0.1^{b}	4.97 ± 0.51	4.59 ± 0.48^{a}	4.70 ± 0.28^{a}

Data represented as means ± SEM. FG: final blood glucose; FBW: final body weight; WC: percentage weight change; LW: liver weight; RLW: relative liver weight. ^{a} $p < 0.05$ vs normal control. ^{b} $p < 0.05$ vs diabetic control. ^{C} $p < 0.05$ vs normal rats treated with vindoline. ^{d} $p < 0.05$ vs normal rats treated with glibenclamide. NC: normal control; NV: normal control treated with vindoline; NG: normal control treated with glibenclamide; DC: diabetic control; DV: diabetic treated with vindoline; DG: diabetic treated with glibenclamide.

Table 2
Effect of vindoline or glibenclamide treatment in T2DM-induced and normal rats.

Test	NC	NV	NG	DC	DV	DG
ALT	34.50 ± 5.13	40.88 ± 2.64[b]	27.88 ± 1.49[b]	161.6 ± 31.56[a]	94.43 ± 17.68[ab]	73.67 ± 13.33[b]
AST	88.13 ± 5.45	103.9 ± 9.05[b]	85.75 ± 8.13[b]	180.9 ± 37.35[a]	105.4 ± 18.98[b]	84.29 ± 13.61[b]
ALP	105.7 ± 9.14[b]	106.3 ± 7.73[b]	90.51 ± 5.33[b]	861.4 ± 132[a]	330.6 ± 51.6 [ab]	469.7 ± 64.2[abc]
LDH	15.36 ± 1.86[b]	19.18 ± 3.14[b]	19.75 ± 2.74[b]	78.81 ± 8.55	73.03 ± 8.85[a]	52.60 ± 9.5[a]
TP(g/L)	50.75 ± 5.55	61.16 ± 8.37[ab]	48.16 ± 3.1[c]	51.30 ± 7.84	43.85 ± 5.23[c]	39.48 ± 5.80[a bc]
Alb(g/L)	27.11 ± 2.65	31.95 ± 3.72[a]	27.40 ± 1.35	28.40 ± 3.07	25.85 ± 2.48[c]	22.58 ± 1.61[abc]
Glob	23.64 ± 1.06	29.21 ± 1.69	20.76 ± 1.45[c]	22.90 ± 2.05	18.00 ± 1.21[c]	16.90 ± 1.66[c]

Data represented as means ± SEM. [a] $p < 0.05$ vs normal control. [b] $p < 0.05$ vs diabetic control. [C] $p < 0.05$ vs normal rats treated with vindoline. NC: normal control; NV: normal control treated with vindoline; NG: normal control treated with glibenclamide; DC: diabetic control; DV: diabetic treated with vindoline; DG: diabetic treated with glibenclamide.

was elevated significantly ($p < 0.05$) in comparison to the diabetic controls. However, we did not observe any substantial differences in insulin levels between the diabetic-glibenclamide group and diabetic controls and diabetic rats treated with vindoline. Neither the percentage body weight change nor the liver weights (expressed as relative liver weights) were significantly modified by vindoline treatment in the diabetic and normal groups when compared to the diabetic controls and the normal untreated group respectively.

3.2. Effects of Vindoline or glibenclamide on serum levels of hepatic enzymes (hepatic function) in T2DM-induced rats

Table 2 illustrates the effects of vindoline or glibenclamide on the activities of liver enzymes (liver function). Induction of T2DM in rats especially the diabetic controls resulted in substantial elevation of serum liver function enzymes (AST, ALT, ALP and LDH) when compared to the non-diabetic groups. Interestingly, vindoline and glibenclamide attenuated the levels of AST, ALT and ALP in diabetic rats in comparison with the diabetic control group ($p < 0.05$) but LDH remained unaltered. Normal rats that received vindoline showed marginally elevated AST levels when compared to the normal controls and normal rats treated with glibenclamide.

The levels of total protein (TP) and albumin in the diabetic controls were significantly higher than the diabetic group treated with glibenclamide ($p < 0.05$). Vindoline did not significantly modify the levels of TP and albumin in T2DM rats when compared to the diabetic controls or the diabetic group. Normal rats treated with vindoline showed significantly increased TP ($P < 0.05$) when compared to all the groups.

3.3. Antioxidant activity of liver homogenates

Presented in Fig. 1 is the hepatic antioxidant status of non-diabetic and diabetic rats. After administration of vindoline and glibenclamide in T2DM, there was a significant improvement of ORAC and SOD enzyme activity while CAT, lipid peroxidation and reduced-GSH were not significantly altered ($p < 0.05$) in comparison with the diabetic control. Additionally, vindoline and glibenclamide administration in non-diabetic rats also improved the activity of SOD and CAT significantly when compared to the normal control group ($p < 0.05$).

3.4. Effects of vindoline or glibenclamide on the levels of triglycerides, TNF-a, IL-10 and IL-6

Fig. 2 shows the effect of vindoline administration on the levels of inflammatory biomarkers and triglycerides. An increase in TNF-α levels was observed in the diabetic controls when compared to the normal control group ($p < 0.05$). Subsequent treatment of T2DM rats with vindoline led to a significant down regulation ($p < 0.05$) of TNF-α levels when compared to the diabetic controls. IL-10 and IL-6 levels in diabetic groups treated with vindoline and glibenclamide were not significantly different from diabetic and normal control groups ($p < 0.05$).

In this study, a marked reduction of serum triglycerides in T2DM treated rats was noted in comparison with the diabetic controls ($p < 0.05$). In addition, the serum triglycerides in the diabetic group treated with vindoline and glibenclamide were restored to near normal levels with no significant differences to normal controls ($p < 0.05$).

3.5. Effect of vindoline or glibenclamide on the histological architecture of the hepatic tissue

Histological assessment of the liver sections of experimental groups revealed the protective effect of vindoline against T2DM-induced tissue damage. As indicated in Table 3 and Fig. 3; all the non-diabetic groups showed normal liver architecture. Induction of diabetes resulted in hepatocellular damage especially in the areas around the central vein, where the cells showed signs of degeneration. However, treatment of the diabetic groups with vindoline or glibenclamide resulted in minimised tissue damage according to the histopathological scoring presented in Table 3. Central vein congestion accompanied by sinusoidal congestion was prominent in the diabetic control group (Fig. 3 (D)) but minimal in the vindoline treated diabetic group as shown in Fig. 3. Dilation and disruption of normal sinusoidal architecture were evident in the diabetic control group and in the diabetic group treated with glibenclamide (arrows in Fig. 3D & F). Moderate infiltration of inflammatory cells was observed in the three diabetic groups.

3.5.1. Histological examination of the pancreas

Effect of vindoline or glibenclamide on the histological architecture of the pancreatic tissue is shown Fig. 4. Plate A, B and C represents normal pancreas with intact islets tissue with no visible signs of injury. The diabetic untreated controls had small islets with vacuolated cells as indicated in plate D1 below. The diabetic rats that were treated with vindoline (plate E) had islets that were larger in size with minimum destruction when compared to the diabetic controls. Administration of glibenclamide to diabetic rats resulted in minimum pancreatic tissue injury when compared to the diabetic controls (plate F).

3.5.2. Quantitative immunohistochemical findings

Fig. 5 illustrates the effect of vindoline and glibenclamide on the expression of apoptotic markers in hepatic tissue of normal and T2DM rats. Quantitative evaluation of images based on different nuclear colour intensities was performed using imageJ software. Caspase 9 was found to be significantly expressed in the diabetic control group at ($p < 0.05$) when compared to all treatment groups. In addition; administration of vindoline and glibenclamide in normal rats resulted in increased expression of caspase 9 ($p < 0.05$) when compared to the normal controls. With respect to the expression of BCL-2, treating T2DM rats with vindoline or glibenclamide did not significantly alter the expression of BCL-2 when compared to the diabetic control group ($p < 0.05$). However the expression of BCL-2 in normal controls was significantly increased when compared to the diabetic control, diabetic

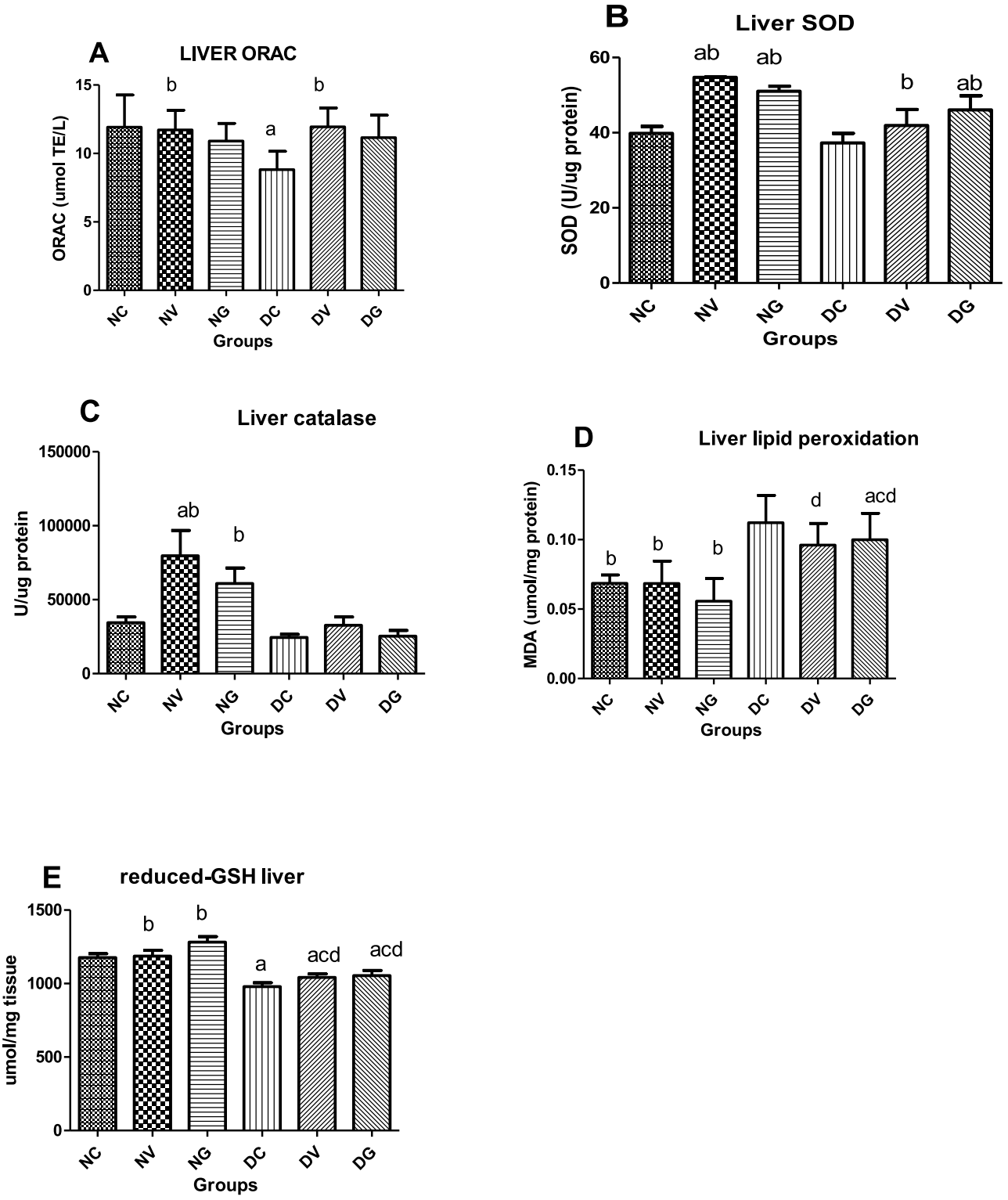


Fig. 1. (A) Oxygen radical antioxidant capacity (ORAC), (B) Superoxide dismutase (SOD), (C) Catalase (CAT) measurements in groups, (D) Lipid peroxidation, (E) Reduced glutathione. Data represented as means ± SEM. [a] $p < 0.05$ vs normal control. [b] $p < 0.05$ vs diabetic control. [C] $P < 0.05$ vs normal rats treated with vindoline. NC: normal control; NV: normal control treated with vindoline; NG: normal control treated with glibenclamide; DC: diabetic control; DV: diabetic treated with vindoline; DG: diabetic treated with glibenclamide.

groups treated with vindoline and glibenclamide ($p < 0.05$).

4. Discussion

The present study aimed to evaluate the effect of vindoline in hepatotoxicity induced by T2DM conditions using glibenclamide as reference drug. Uncontrolled hyperglycemia in DM is the hallmark of free radical formation implicated in the modification of structures and functions of macromolecules contributing to early development of diabetic complications [33,34]. Antidiabetic therapy must therefore

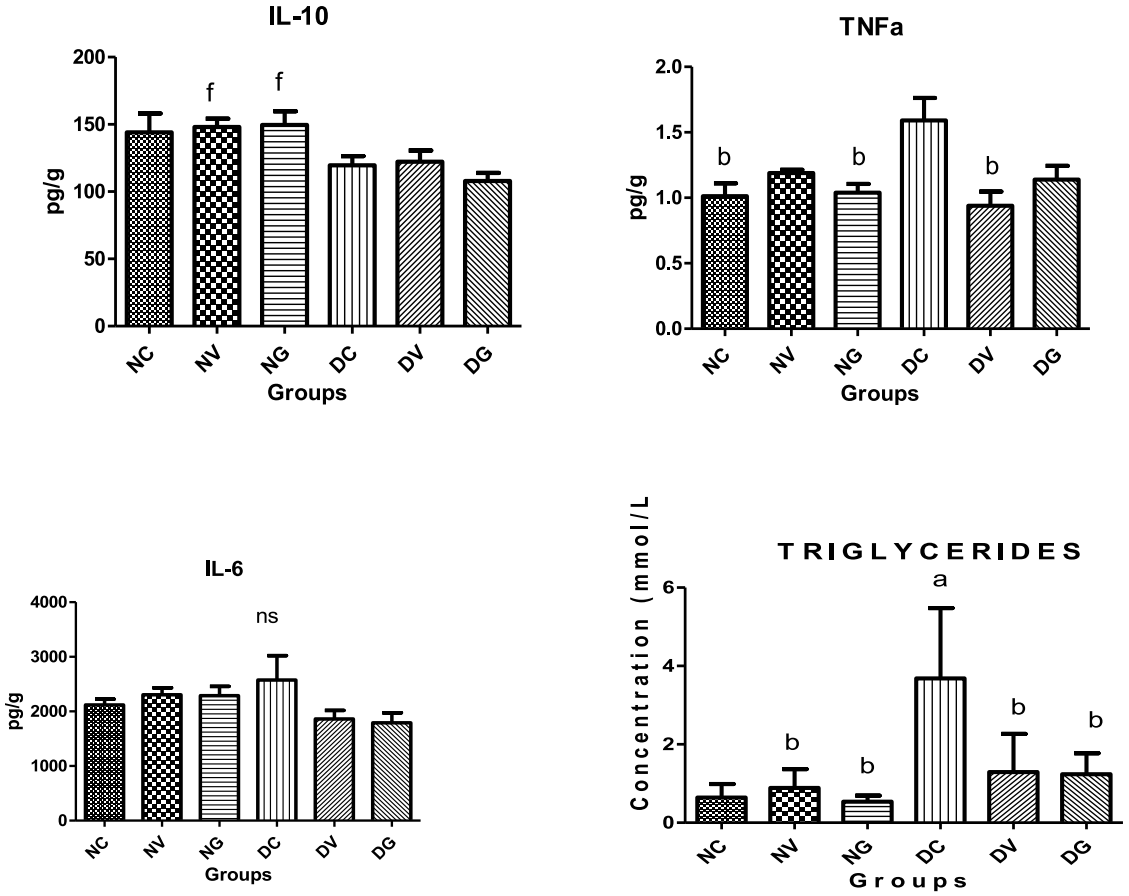


Fig. 2. Effects of vindoline or glibenclamide on the levels of inflammatory cytokines: TNF-α, IL-10 and IL-6. Data represented as means ± SEM. [a] $p < 0.05$ vs normal control. [b] $p < 0.05$ vs diabetic control. [C] $P < 0.05$ vs normal rats treated with vindoline. [d] $p < 0.05$ vs normal rats treated with glibenclamide. [f] $p < 0.05$ vs diabetic rats treated with glibenclamide. [ns] $p < 0.5$ non-significant change among all groups. NC: normal control; NV: normal control treated with vindoline; NG: normal control treated with glibenclamide; DC: diabetic control; DV: diabetic treated with vindoline; DG: diabetic treated with glibenclamide.

Table 3
Histopathology score in hepatic tissue.

	NC	NV	NG	DC	DV	DG
	Liver					
Hepatocyte injury	0	0	0	2	1	1
Central vein congestion	0	0	0	2	1	1
Sinusoidal congestion	0	0	0	2	1	1
Sinusoidal dilation	0	0	0	2	0	1
Infiltration of inflammatory cells	0	0	0	1	1	1

Hepatic injury was scored as follows: o: absence of cell damage in > 80% of the tissue; 1: damage < 30% of the tissue; 2: damage between 30–50% of tissue; 3: necrosis > 50% of tissue. NC: normal control; NV: normal control treated with vindoline; NG: normal control treated with glibenclamide; DC: diabetic control; DV: diabetic treated with vindoline; DG: diabetic treated with glibenclamide.

aim to maintain normoglycemia in addition to delay or prevention of tissue injury [35]. Natural products are promising agents that could be effective and safer in ameliorating diabetic complications [36]. Vindoline is an indole alkaloid derived from the leaves of *C.roseus* and has been reported to stimulate the release of insulin from pancreatic beta cells [37].

The results of this study showed a significant reduction in fasting blood glucose levels in diabetic rats following treatment with vindoline. It appeared that administration of glibenclamide in diabetic rats exhibited lesser effects on fasting glucose level than vindoline which suggests that vindoline may possess a better insulin secretory effect than glibenclamide. We presumed that vindoline's mechanism of action was through stimulation of existing pancreatic β cells to secrete insulin. Our findings are in agreement with the results reported by Yao et al [37] who observed a marked reduction in blood glucose levels in T2DM obese model. One of the side-effects of compounds that enhance insulin excretory effect is hypoglycaemia. Based on our current findings; administration of vindoline in non-diabetic rats did not induce hypoglycaemia probably because the dosage administered was lower and tolerable [37,38].

Insulin deregulation is predictive of abnormal cofactors like hepatic insulin resistance, hyperglycemia and β-cell dysfunction which modulate metabolic derangements [10]. Vindoline administration in T2DM rats led to the elevation of insulin, the levels were higher than those observed in the diabetic group treated with glibenclamide. Increased insulin levels may have been due to its previously reported Kv2.1 inhibitory effect which reduces the voltage-dependent outward potassium current resulting in insulin secretion by pancreatic β-cells [37]. Despite the increased insulin level in the serum of both diabetic treated rats, hyperglycemia was still present thus indicating resistance to the action of insulin. In normal rats, vindoline administration did not stimulate secretion of insulin, suggesting that vindoline may not have insulotropic effects in normoglyecemic condition which is also in agreement with Yao et al [37] findings.

It has been shown that DM patients suffer from severe weight loss as a result of increased catabolism of fats and proteins in the skeletal muscle in response to the deranged carbohydrate metabolism [39]. The current study showed diminished body weight in all diabetic groups in which vindoline and glibenclamide both failed to ameliorate weight loss. Such result may be an indication that glycogenolysis, lipolysis and proteolysis were still going on in order to compensate for the lack of

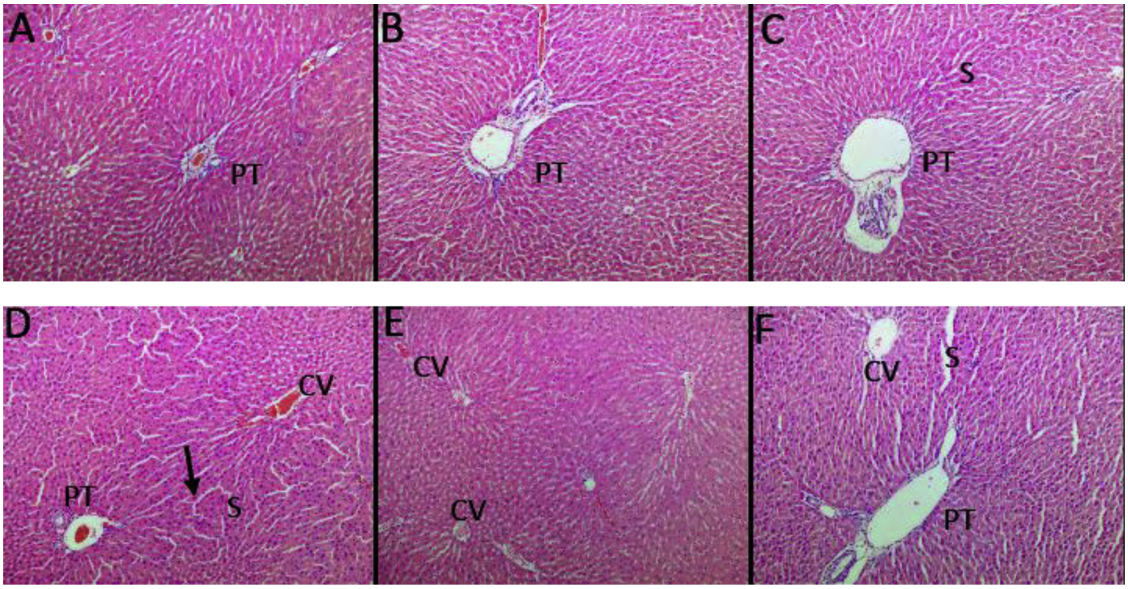


Fig. 3. represents the haematoxylin and eosin stained liver sections (X100). A: normal control; B: normal control treated with vindoline; C: normal control treated with glibenclamide; D: diabetic control; E: diabetic treated with vindoline; F: diabetic treated with glibenclamide. PT: portal tract; CV: central vein; S: sinusoids; arrows indicate sinusoidal dilation and disordered arrangement of sinusoids.

glucose in the cells [40]. Liver hypertrophy is a common observation in diabetic animals attributed to increased free fatty acids pools in the hepatocytes as a consequence of abnormal insulin metabolism [32]. All diabetic groups in our study had increased relative liver weight when compared to the normal groups. Treatment with vindoline and glibenclamide in diabetic rats did not significantly reverse liver hypertrophy.

Hepatic enzymes which include amino transferases (AST and ALT) and alkaline phosphatase (ALP) are the first line of markers used to determine hepatic injury. In a state of hepatocellular damage, these enzymes leak into the serum resulting in elevated levels. Elevated serum transaminases are commonly observed in diabetics as a result of

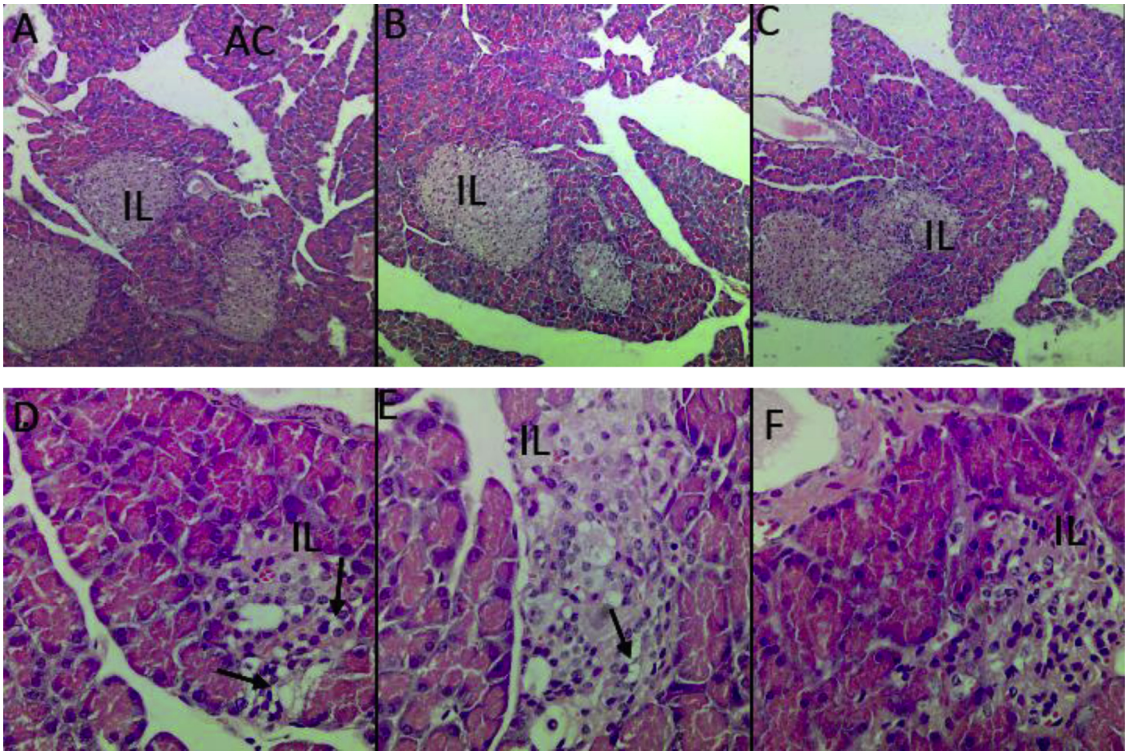
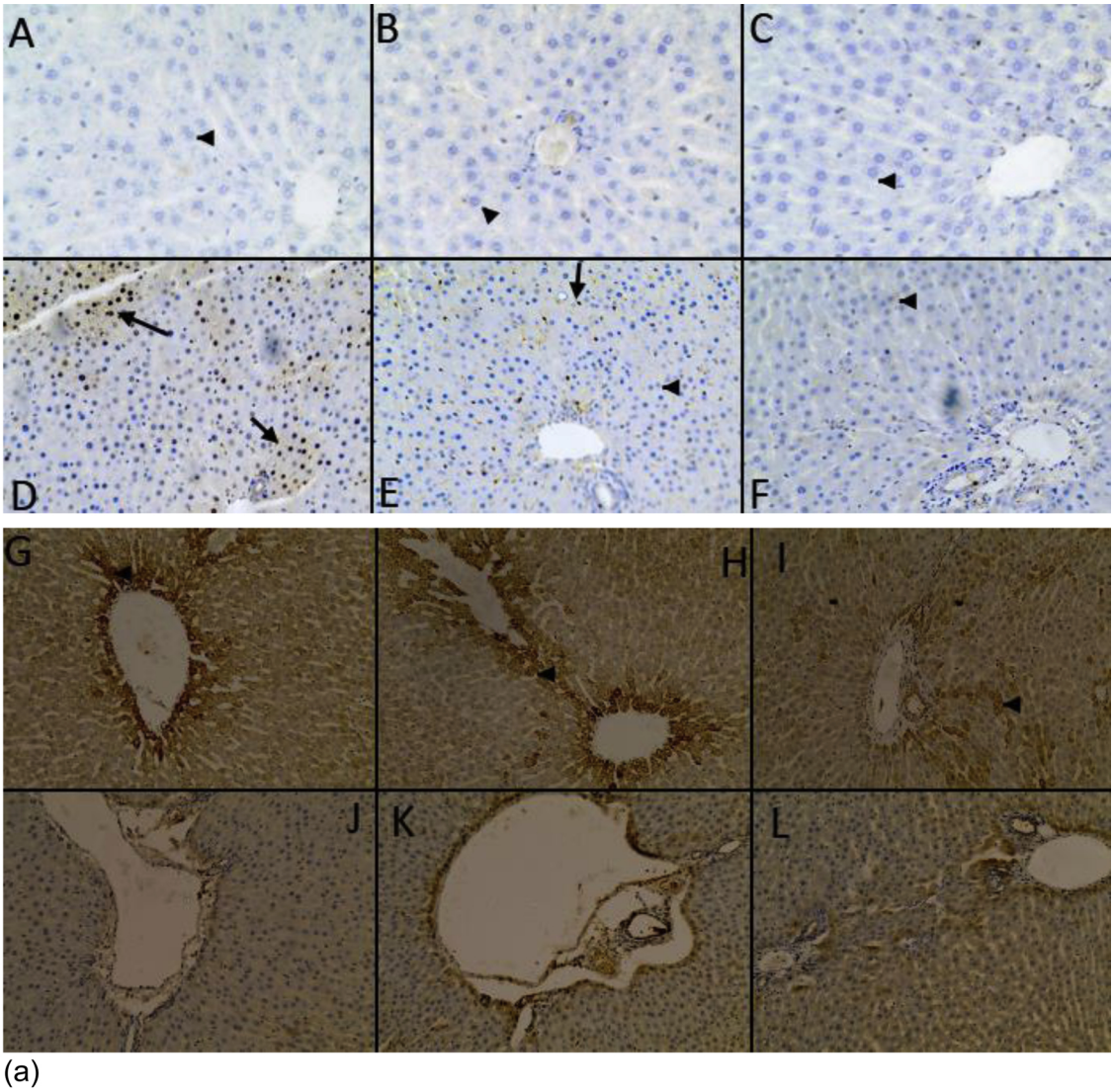


Fig. 4. Histopathological changes in the pancreatic tissue of treated and non-treated diabetic and non-diabetic rats. Plate A (normal control) shows normal islets tissue (IL) with well-defined borders, surrounded by the exocrine acinus cell (AC). Plates B (normal-treated with vindoline) and C (normal treated with glibenclamide) show normal islets (IL). Plate D (diabetic control) shows loss of the normal size and compactness of the islets, vacuolated degenerated islets (black arrows). Treated islets with vindoline were smaller in size when compared to the normal but relatively larger in size than the diabetic controls. The Islets showed a degree of vacuolation (arrows in Plate E) with slight regeneration. Glibenclamide treatment in diabetic rats (Plate F) showed few vacuolated islets and minimum loss of islet architecture.



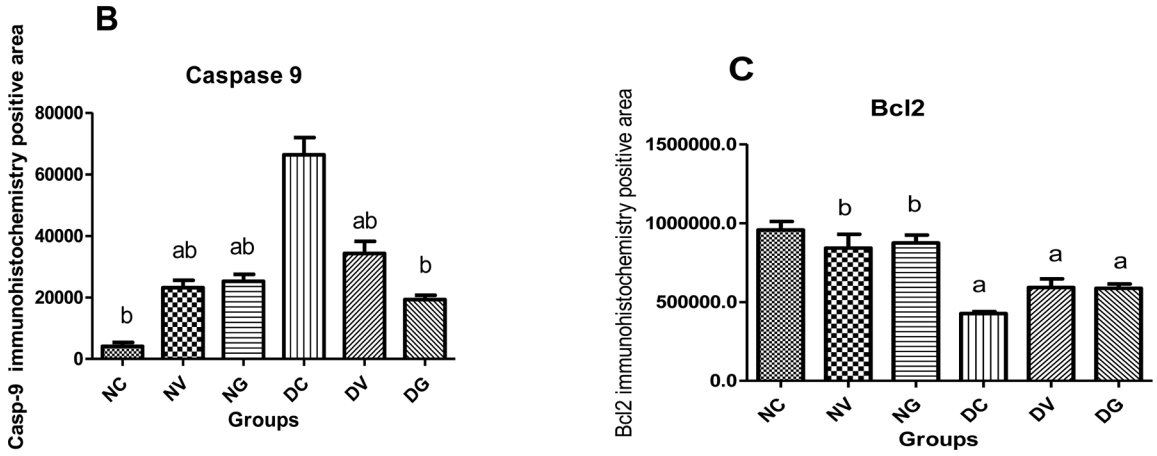


Fig. 5. Pictorial and quantitative immunohistochemical representation of apoptotic markers of the liver sections labelled with anti-Bcl-2 and anti-caspase 9. Data represented as means ± SEM. [a] $p < 0.05$ vs normal control. [b] $p < 0.05$ vs diabetic control. [C] $p < 0.05$ vs normal rats treated with vindoline. [f] $p < 0.05$ vs diabetic rats treated with glibenclamide. Plate A–F represents caspase 9 marker intensities; Plate G–L represents Bcl-2 marker intensities. A/G = (NC): normal control; B/H = (NV) = normal control treated with vindoline; C/I = (NG) = normal control treated with glibenclamide; D/J = DC = diabetic control; E/K = DV = diabetic treated with vindoline; F/L = DG = diabetic treated with glibenclamide; arrows indicate high immunoreactive cells in anti-caspase 9 pictographs, arrow heads represents negative immunoreactivity in anti-caspase 9 pictographs. In Bcl-2 pictographs; arrow heads indicate high immunoreactivity.

functional disturbances of hepatocyte membranes [41–43]. Upon oral administration of vindoline and glibenclamide in T2DM rats, the serum levels of ALT, ALP and AST reduced significantly while LDH remained unchanged when compared to the diabetic untreated group. These results may indicate comparable hepatoprotective effect of vindoline and glibenclamide probably brought about by increased hepatocyte membranal stability and hepatocellular regeneration. No significant alterations were seen in normal rats treated with vindoline when compared to the normal controls and normal rats treated with glibenclamide.

It is known that hyperglycaemia stimulates the breakdown of structural proteins directly affecting the synthesis and secretion of vital proteins like albumin [36,44]. Albumin is a major transporter protein that carries analytes during various metabolic processes [45]. In the present study, total protein concentration was lower in the diabetic groups treated with vindoline (not significantly) and glibenclamide (significantly) when compared to the untreated diabetic control. This decrease could have been attributed to the increased binding of the vindoline and glibenclamide components to serum albumins. This result is in agreement with the results obtained by Iweala and Okeke [46] who reported low levels of total protein in rats treated with *C.roseus* and chlorpropamide. Unexpectedly, the total protein levels were high in the diabetic controls without any significant difference when compared to the normal controls. It is logical to argue that the high total protein levels in the diabetic controls could have been falsely increased as a result of dehydration due to polyuria [47,48]. In the vindoline treated normal controls, a marked significant increase in the total protein was seen when compared with the normal control and the glibenclamide treated normal rats.

It is well established that persistent hyperglycemia is closely linked to oxidative stress [49]. Oxidative stress is the underlying mechanism in the development of diabetic complications via the excessive formation of ROS and/ insufficient production of antioxidants [11,50]. In order to evaluate if vindoline could decrease/ prevent oxidative damage, we measured the oxygen radical absorbance capacity (ORAC), SOD and CAT activities, lipid peroxidation and reduced-GSH levels in the liver homogenates. The ORAC assay is performed to assess the ability of biological samples to withstand the deleterious effects of excess free radicals. The ability of the homogenates to scavenge oxygen radical was augmented significantly to near normal in the T2DM rats treated with vindoline more than the diabetic controls. Glibenclamide's effect on ORAC in diabetic rats was lower than that of vindoline suggesting that vindoline may prevent cellular damage through its strong free radical reduction capacity [50].

Superoxide dismutase, CAT and GSH are endogenous antioxidants that work hand in hand to combat free radical toxicity in tissues. SOD is an antioxidant enzyme responsible for the dismutation of the unstable superoxide radicals into H_2O_2, while catalase catalyses the decomposition of H_2O_2 into inert water and oxygen [45]. GSH is an antioxidant defence molecule with powerful scavenging and detoxifying potential against hydroxyl anion and oxygen radical [51]. Excessive free radical build-up has been implicated in the initiation peroxidation of cell membrane lipids. Peroxidation of membranal lipids destroys the structural and functional integrity of the cell causing faulty permeability which is harmful to cellular organelles [52].

In our findings; we observed obvious decrease in the activities of these enzymes as well as in the level of GSH in the diabetic controls. Interestingly, administration of vindoline and glibenclamide in diabetic rats significantly improved the hepatic antioxidant status as evidenced by elevated activities of SOD thus increasing protection against oxidative injury. The same increase was observed in non-diabetic groups that received glibenclamide and vindoline in comparison with the non-treated controls. On the other hand; CAT activity and reduced-GSH level in the vindoline treated diabetic group increased but not significantly when compared to the diabetic controls and diabetic-glibenclamide treated group. Treatment of diabetic rats with glibenclamide or vindoline did not significantly reduce the formation of by-products of lipid peroxidation suggesting that their antioxidant effect only boosts the first line of free radical defence antioxidants [52]. The antioxidant effect exhibited by vindoline in this study shows an alternative therapeutic effect of vindoline that could be useful in the treatment of diabetes mellitus [49,53].

Various studies have indicated the important role inflammation plays in the pathogenesis of insulin resistance, T2DM and its complications [54]. Inflammation is an adaptive response elicited by the body following tissue injury in attempts to reverse injury [55]. The increased release of inflammatory cytokines such as TNF-a, IL-6 and decreased production of IL-10 in DM has been documented in diabetic models [34]. We observed that the levels of IL-10-an anti-inflammatory cytokine were significantly higher in the normal rats treated with vindoline or glibenclamide when compared to the diabetic group treated with glibenclamide. Vindoline administration in diabetic rats did not significantly affect the levels of IL-10.

Depending on the tissue and metabolic state; IL-6 exists as both a pro and anti-inflammatory cytokine [56]. According to our results, IL-6 levels in the hepatic homogenates were decreased in diabetic rats treated with vindoline and glibenclamide although the results were not significant when compared to the diabetic control. This result may indicate that vindoline and glibenclamide could possibly safeguard the hepatic tissue by suppressing the release of IL-6 by kupffer cells in hyperglycaemic milleu. Due to the complexity of IL-6 metabolism, in the non-diabetic groups' IL-6 levels were not significantly different from the diabetic control. This finding in the normal rats may be due to the beneficial roles (anti-inflammatory) exerted by IL-6 [57]. A positive correlation between TNF-α and hepatic injury in diabetes has been documented mainly because TNF-α promotes insulin resistance that leads to severe hyperglycemia and finally oxidative injury [58]. Assessment of TNF-α demonstrated elevated levels in the diabetic control group while, oral administration of vindoline evidently decreased TNF-α levels. This result suggests better potential immunomodulatory effect of vindoline in comparison to glibenclamide which may be of therapeutic relevance in preventing and/ retarding the onset of hepatic tissue injury elicited by adverse inflammatory responses [59].

Abnormal fatty acid metabolism in diabetes mellitus is associated with hypertriglyceridemia which is a risk factor for the development of cardiovascular related diseases (CVDs) and NAFLD [60,61]. Lipoprotein lipase is an enzyme that normally acts by hydrolysing triglycerides in the presence of insulin. However in DM, its activation is denied due to insulin deficiency resulting in hypertriglyceridemia [61,62]. Based on our results, it is evident that treatment of diabetic rats with vindoline decreased serum triglyceride levels when compared to the diabetic controls. Vindoline and glibenclamide administration in T2DM rats restored the triglyceride levels to near normal levels as the non-diabetic controls. The reduction of the serum triglycerides in the diabetic treated groups may have been due the increased activity of lipoprotein lipase thus increasing the hydrolysis of triglycerides [40]. This observation implies that vindoline may reduce the cardiovascular and NAFLD risks associated with diabetes [63].

Our histopathological findings revealed that induction of T2DM resulted in hepatic tissue damage which was confirmed by changes in the tissue architecture. Liver section in diabetic rats demonstrated severe mild leukocyte infiltration, central vein congestion, sinusoidal dilation and congestion and hepatocyte vaculoation. These findings were in agreement with the findings of Aldahmash et al [64] who reported similar changes in DM mice. Administration of vindoline showed appreciable improvements to these abnormal alterations revealed by close to normal sinusoidal pattern and reduced congestion of the central vein. Administration of glibenclamide in diabetic rats could not reverse the dilation of sinusoids, which indicated vindoline's more robust effect in restoring diabetic liver pathology. Consequently, it was clearly observed that induction of STZ in conjunction with the 10% fructose water resulted in the destruction of insulin secreting β cells of the pancreas as shown in Fig. 4 in plate D1 and D2. The pancreas of

vindoline treated rats showed improvement in the size and islet morphology when compared to the diabetic control and the diabetic-glibenclamide treated groups. Our results largely highlight vindoline may not only stimulate insulin secretion but also protect the intact functional β cells from STZ-induced destruction [37].

Oxidative stress together with adverse cytokine responses elicit the activation of apoptosis in biological systems [65]. Bcl-2 is an important protein that regulates apoptosis by preventing cell death [66]. According to the hepatic immunohistochemical reports, there was downregulation of Bcl-2 protein expression in the diabetic control rats. Oral administration of vindoline in diabetic rats did not significantly increase the expression of Bcl-2 when compared to the diabetic control group. However, there was a marked downregulation of caspase 9 in the hepatic tissue of diabetic rats treated with vindoline when compared to the diabetic controls. Unlike Bcl-2, caspase 9 is a pro-apoptotic protein which activates executioner-caspase 3 thus decreasing survival chances of the cell. Enhanced expression of caspase 9 in diabetic control rats in this study could indicate failed control of apoptosis in the mitochondrial pathway [65,67]. Therefore, vindoline may be a valuable therapeutic approach in the prevention of hepatic cell death in diabetes mellitus.

5. Conclusion

In conclusion, our experimental findings suggest that vindoline as a plant-derived product may be of great relevance in the treatment of diabetes mellitus and its complications owing to its anti-hyperglycemic, antihyperlipidemic, anti-inflammatory and antioxidant activities. Further experimental studies are recommended to improve vindoline's delivery and absorption so as to increase its therapeutic effect.

Competing interest

The authors declare no competing interest.

Funding

This research work was funded by the University Research Fund (RJ-23) and the National Research Fund Grant (NRF-RO22) awarded to Professor O.O. Oguntibeju for which authors are grateful. The funding bodies had no role in the research design of the study, analysis and manuscript writing.

Acknowledgements

The authors wish to thank Mr Fanie Rautenbach of the Oxidative Stress Research Centre at Cape Peninsula University of Technology for his remarkable assistance with the analysis of oxidative stress biomarkers. We thank Ms Fadia Alexandra of Cape Peninsula University of Technology for assisting with blood chemistry analysis.

References

[1] H. Ahmadieh, S.T. Azar, Liver disease and diabetes: association, pathophysiology, and management, Diabetes Res. Clin. Pract. 104 (2014) 53–62, https://doi.org/10.1016/j.diabres.2014.01.003.
[2] World Health Organization, Global Report on Diabetes Vol. 978 Isbn., 2016 88. doi:ISBN 978 92 4 156525 7.
[3] WHO, Global Status Report on Noncommunicable Diseases 2014 vol. 176, World Health, 2014 doi:ISBN 9789241564854.
[4] A. Mouralidarane, J.A. Oben, J. Soeda, Pathophysiology and clinical management of non-alcoholic fatty liver disease, Medicine (Baltimore) 39 (2011) 592–596, https://doi.org/10.1016/j.mpmed.2011.07.014.
[5] D. Garcia-Compean, Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management, World J. Gastroenterol. 15 (2009) 280, https://doi.org/10.3748/wjg.15.280.
[6] S. Calanna, R. Scicali, A. Di Pino, F.K. Knop, S. Piro, A.M. Rabuazzo, F. Purrello, Alpha- and beta-cell abnormalities in haemoglobin A1c-defined prediabetes and type 2 diabetes, Acta Diabetol. 51 (2014) 567–575, https://doi.org/10.1007/s00592-014-0555-5.
[7] S. de M. Bandeira, L.J.S. da Fonseca, G. da S. Guedes, L.A. Rabelo, M.O.F. Goulart, S.M.L. Vasconcelos, Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus, Int. J. Mol. Sci. 14 (2013) 3265–3284, https://doi.org/10.3390/ijms14023265.
[8] M. Torres, J. Canal, C. Perez, Oxidative stress in normal and diabetic rats, Physiol. Res. 48 (1999) 203–208 http://www.biomed.cas.cz/physiolres/pdf/48/48_203.pdf.
[9] J. Mohamed, A.H. Nazratun Nafizah, A.H. Zariyantey, S.B. Budin, Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation, Sultan Qaboos Univ. Med. J. 16 (2016) e132–e141, https://doi.org/10.18295/squmj.2016.16.02.002.
[10] D.E. Francés, M.T. Ronco, J.A. Monti, P.L. Ingaramo, G.B. Pisani, J.P. Parody, J.M. Pellegrino, P.M. Sanz, M.C. Carrillo, C.E. Carnovale, Hyperglycemia induces apoptosis in rat liver through the increase of hydroxy, radical: new insights into the insulin effect, J. Endocrinol. 205 (2010) 187–200, https://doi.org/10.1677/JOE-09-0462.
[11] G.K. Xu, X.Y. Qin, G.K. WANG, G.Y. Xie, X. Sen Li, C.Y. Sun, B.L. Liu, M.J. Qin, Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of Bombax ceiba leaves in high-fat-diet- and streptozotocin-induced Type 2 diabetic rats, Chin. J. Nat. Med. 15 (2017) 168–177, https://doi.org/10.1016/S1875-5364(17)30033-X.
[12] C. Town, R.T. Erasmus, D.J. Soita, M.S. Hassan, E. Blanco-blanco, Z. Vergotine, A.P. Kengne, T.E. Matsha, Research High Prevalence of Diabetes Mellitus and Metabolic Syndrome in a South African Coloured Population : Baseline Data of a Study in, 102, (2012), pp. 841–844, https://doi.org/10.7196/SAMJ.5670.
[13] A.H. Hassan, M.I. Sule, A.M. Musa, K.Y. Musa, M.S. Abubakar, A. Hassan, Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria Department of Biological Science, Kaduna State University, Kaduna, Afr. J. Tradit. Complement. Altern. Med. 9 (2012) 250–255.
[14] S. Bhatia, R. Shukla, S. Venkata Madhu, J. Kaur Gambhir, K. Madhava Prabhu, Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy, Clin. Biochem. 36 (2003) 557–562, https://doi.org/10.1016/S0009-9120(03)00094-8.
[15] L. Almagro, F. Fernández-Pérez, M.A. Pedreño, Indole alkaloids from Catharanthus roseus: bioproduction and their effect on human health, Molecules 20 (2015) 2973–3000, https://doi.org/10.3390/molecules20022973.
[16] A. Natarajan, K. Syed Zameer Ahmed, S. Sundaresan, A. Sivaraj, K.S. devi, B. Senthil Kumar, Effect of Aqueous Flower Extract of Catharanthus roseus on Alloxan Induced Diabetes in Male Albino Rats, (2012).
[17] W.M. Al-Shaqha, M. Khan, N. Salam, A. Azzi, A.A. Chaudhary, Anti-diabetic potential of Catharanthus roseus Linn. and its effect on the glucose transport gene (GLUT-2 and GLUT-4) in streptozotocin induced diabetic wistar rats, BMC Complement. Altern. Med. 15 (2015) 1–8, https://doi.org/10.1186/s12906-015-0899-6.
[18] S.H. Tiong, C.Y. Looi, H. Hazni, A. Arya, M. Paydar, W.F. Wong, S.C. Cheah, M.R. Mustafa, K. Awang, Antidiabetic and antioxidant properties of alkaloids from Catharanthus roseus (L.) G. Don, Molecules 18 (2013) 9770–9784, https://doi.org/10.3390/molecules18089770.
[19] A.P. Attanayake, K.A.P.W. Jayatilaka, C. Pathirana, L.K.B. Mudduwa, Antihyperglycaemic, antihyperlipidaemic and β cell regenerative effects of Spondias pinnata (Linn. f.) Kurz. bark extract on streptozotocin induced diabetic rats, Eur. J. Integr. Med. 6 (2014) 588–596, https://doi.org/10.1016/j.eujim.2014.03.010.
[20] M. Jayanthi, N. Sowbala, G. Rajalakshmi, U. Kanagavalli, V. Sivakumar, Study of anti hyperglycemic effect of Catharanthus roseus in alloxan induced diabetic rats, Int. J. Pharm. Pharm. Sci. 2 (2010) 114–116.
[21] S.H. Tiong, C.Y. Looi, A. Arya, W.F. Wong, H. Hazni, M.R. Mustafa, K. Awang, Vindogentianine, a hypoglycemic alkaloid from Catharanthus roseus (L.) G. Don (Apocynaceae), Fitoterapia 102 (2015) 182–188, https://doi.org/10.1016/j.fitote.2015.01.019.
[22] S.N. Singh, P. Vats, S. Suri, R. Shyam, M.M. Kumria, S. Ranganathan, K. Sridharan, Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats, J. Ethnopharmacol. 76 (2001) 269–277 http://www.ncbi.nlm.nih.gov/pubmed/11448549.
[23] R. Blakytny, J.J. Harding, Glycation (non-enzymic glycosylation) inactivates glutathione reductase, Biochem. J. 288 (Pt 1) (1992) 303–307 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1132114&tool=pmcentrez&rendertype=abstract.
[24] S. Nammi, M.K. Boini, S.D. Lodagala, R.B.S. Behara, The juice of fresh leaves of Catharanthus roseus Linn. reduces blood glucose in normal and alloxan diabetic rabbits, BMC Complement. Altern. Med. 3 (2003) 2–5, https://doi.org/10.1186/1472-6882-3-4.
[25] K. Rasineni, R. Bellamkonda, S.R. Singareddy, S. Desireddy, Abnormalities in carbohydrate and lipid metabolisms in high-fructose dietfed insulin-resistant rats: amelioration by Catharanthus roseus treatments, J. Physiol. Biochem. 69 (2013) 459–466, https://doi.org/10.1007/s13105-013-0233-z.
[26] K.L. De Angelis, I.A. Cestari, J. Barp, P. Dall'Ago, T.G. Fernandes, P.I. de Bittencourt, A. Belló-Klein, A.A. Belló, S. Llesuy, M.C. Irigoyen, Oxidative stress in the latissimus dorsi muscle of diabetic rats, Braz. J. Med. Biol. Res. 33 (2000) 1363–1368, https://doi.org/10.1590/S0100-879X2000001100016.
[27] H.B.T.-M, E. Aebi, 13] Catalase in vitro, Oxyg. Radicals Biol. Syst, Academic Press, 1984, pp. 121–126, https://doi.org/10.1016/S0076-6879(84)05016-3.
[28] N. Crosti, J. Bajer, M. Gentile, G. Resta, A. Serra, Catalase and glutathione peroxidase activity in cells with trisomy 21, Clin. Genet. 36 (2008) 107–116, https://doi.

org/10.1111/j.1399-0004.1989.tb03172.x.
[29] D.J. Jollow, J.R. Mitchell, N. Zampaglione, J.R. Gillette, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-Bromobenzene oxide as the hepatotoxic metabolite, Pharmacology 11 (1974) 151–169, https://doi.org/10.1159/000136485.
[30] T. Tug, F. Karatas, S.M. Terzi, Antioxidant vitamins (A, C and E) and malondialdehyde levels in acute exacerbation and stable periods of patients with chronic obstructive pulmonary disease, Clin. Invest. Med. 27 (2004) 123–128 http://europepmc.org/abstract/MED/15305803.
[31] B. Ou, M. Hampsch-Woodill, R.L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, J. Agric. Food Chem. 49 (2001) 4619–4626, https://doi.org/10.1021/jf010586o.
[32] O.O. Oguntibeju, S. Meyer, Y.G. Aboua, M. Goboza, Hypoxis hemerocallidea significantly reduced hyperglycaemia and hyperglycaemic-induced oxidative stress in the liver and kidney tissues of streptozotocin-induced diabetic male wistar rats, Evid. Complement. Alternat. Med. 2016 (2016), https://doi.org/10.1155/2016/8934362.
[33] M. a Mcanuff, F.O. Omoruyi, E.Y.S. a Morrison, H.N. Asemota, Changes in some liver enzymes in streptozotocin-induced diabetic rats fed sapogenin extract from bitter yam (Dioscorea polygonoides) or commercial diosgenin cambios en algunas enzimas hepáticas en ratas con diabétes inducida mediante estreptozotocina, West Indian Med. J. 54 (2005) 97–101.
[34] O.R. Ayepola, N.N. Chegou, N.L. Brooks, O.O. Oguntibeju, Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses, BMC Complement. Altern. Med. 13 (2013) 1–9, https://doi.org/10.1186/1472-6882-13-363.
[35] R.D. Wilson, S. Islam, M.S. Islam, S. Islam, Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes, Pharmacol. Rep. 64 (2012) 129–139, https://doi.org/10.1016/S1734-1140(12)70739-9.
[36] A.K. Sharma, R. Gupta, Anti-hyperglycemic activity of aqueous extracts of some medicinal plants on wistar rats, J. Diabetes Metab. 08 (2017), https://doi.org/10.4172/2155-6156.1000752.
[37] X.G. Yao, F. Chen, P. Li, L. Quan, J. Chen, L. Yu, H. Ding, C. Li, L. Chen, Z. Gao, P. Wan, L. Hu, H. Jiang, X. Shen, Natural product vindoline stimulates insulin secretion and efficiently ameliorates glucose homeostasis in diabetic murine models, J. Ethnopharmacol. 150 (2013) 285–297, https://doi.org/10.1016/j.jep.2013.08.043.
[38] D. Sola, L. Rossi, G.P.C. Schianca, P. Maffioli, M. Bigliocca, R. Mella, F. Corlianò, G. Paolo Fra, E. Bartoli, G. Derosa, Sulfonylureas and their use in clinical practice, Arch. Med. Sci. 11 (2015) 840–848, https://doi.org/10.5114/aoms.2015.53304.
[39] N. Møller, K.S. Nair, Diabetes and protein metabolism, Diabetes 57 (2008) 3–4, https://doi.org/10.2337/db07-1581.
[40] R.A. Adisa, M.I. Choudhary, O.O. Olorunsogo, Hypoglycemic activity of Buchholzia coriacea (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice, Exp. Toxicol. Pathol. 63 (2011) 619–625, https://doi.org/10.1016/j.etp.2010.05.002.
[41] D.L. Regidor, C.P. Kovesdy, R. Mehrotra, M. Rambod, J. Jing, C.J. McAllister, D. Van Wyck, J.D. Kopple, K. Kalantar-Zadeh, Serum alkaline phosphatase predicts mortality among maintenance hemodialysis patients, J. Am. Soc. Nephrol. 19 (2008) 2193–2203, https://doi.org/10.1681/ASN.2008010014.
[42] P. Visweswara Rao, K. Madhavi, M. Dhananjaya Naidu, S.H. Gan, Rhinacanthus nasutus improves the levels of liver carbohydrate, protein, glycogen, and liver markers in streptozotocin-induced diabetic rats, Evid. Complement. Alternat. Med. 2013 (2013), https://doi.org/10.1155/2013/102901.
[43] Esmaiel Amraie, Masome Khosravi Farsani, Leila Sadeghi, Tayaba Naim Khan, Vahid Yousefi Babadi, Zohrab Adavi, The effects of aqueous extract of alfalfa on blood glucose and lipids in alloxan-induced diabetic rats, Interv. Med. Appl. Sci. 7 (2015) 124–128, https://doi.org/10.1556/1646.7.2015.3.7.
[44] A. Zarei, G. Vaezi, A.A. Malekirad, M. Abdollahi, Effects of ethanol extract of Salvia hydrangea on hepatic and renal functions of streptozotocin-induced diabetic rats, Avicenna J. Phytomed. 5 (2015) 138–147.
[45] E.I. Omodanisi, Y.G. Aboua, O.O. Oguntibeju, R.M. Lamuela-Raventós, Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of moringa oleifera in diabetes-induced nephrotoxic male wistar rats, Molecules 22 (2017) 1–16, https://doi.org/10.3390/molecules22040439.
[46] J.E.E. Iweala, U.C. Okeke, Comparative study of the hypoglycemic and biochemical effects of Catharanthus roseus (Linn) g. apocynaceae (Madagascar periwinkle) and Chlopropamide (diabenese) on alloxan-induced diabetic rats, Biokemistry 17 (2005) 149–156.
[47] M. Thomson, Antidiabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin- induced diabetic rats, Int. J. Diabetes Metab. (2007).
[48] M. Hotta, R. Ohwada, T. Akamizu, T. Shibasaki, K. Kangawa, Chapter twenty-four - therapeutic potential of ghrelin in restricting-type anorexia nervosa, in: M. Kojima, K.B.T.-M, E. Kangawa (Eds.), Ghrelin, Academic Press, 2012, pp. 381–398, , https://doi.org/10.1016/B978-0-12-381272-8.00024-6.
[49] P. Martín-Gallán, A. Carrascosa, M. Gussinyé, C. Domínguez, Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications, Free Radic. Biol. Med. 34 (2003) 1563–1574, https://doi.org/10.1016/S0891-5849(03)00185-0.
[50] V. Sivakumar, M. Dhana Rajan, Antioxidant effect of Tinospora cordifolia extract in alloxan-induced diabetic rats, Indian J. Pharm. Sci. 72 (2010) 795, https://doi.org/10.4103/0250-474X.84600.
[51] N.K. Nazaroglu, A. Sepici-Dincel, N. Altan, The effects of sulfonylurea glyburide on superoxide dismutase, catalase, and glutathione peroxidase activities in the brain tissue of streptozotocin-induced diabetic rat, J. Diabetes Complications 23 (2009) 209–213, https://doi.org/10.1016/j.jdiacomp.2007.09.001.
[52] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, Alexandria J. Med. (2017) 1–7, https://doi.org/10.1016/j.ajme.2017.09.001.
[53] S.C. Mohan, T. Anand, G.S. Priyadharshini, V. Balamurugan, Research Article GC-MS Analysis of Phytochemicals and Hypoglycemic Effect of, 31, (2015), pp. 123–128.
[54] N.G. Cruz, L.P. Sousa, M.O. Sousa, N.T. Pietrani, A.P. Fernandes, K.B. Gomes, The linkage between inflammation and Type 2 diabetes mellitus, Diabetes Res. Clin. Pract. 99 (2013) 85–92, https://doi.org/10.1016/j.diabres.2012.09.003.
[55] R.M. Pollack, M.Y. Donath, D. LeRoith, G. Leibowitz, Anti-inflammatory agents in the treatment of diabetes and its vascular complications, Diabetes Care 39 (2016) S244–S252, https://doi.org/10.2337/dcS15-3015.
[56] J. Scheller, A. Chalaris, D. Schmidt-Arras, S. Rose-John, The pro- and anti-inflammatory properties of the cytokine interleukin-6, Biochim. Biophys. Acta - Mol. Cell Res. 1813 (2011) 878–888, https://doi.org/10.1016/j.bbamcr.2011.01.034.
[57] D. Schmidt-Arras, S. Rose-John, IL-6 pathway in the liver: from physiopathology to therapy, J. Hepatol. 64 (2016) 1403–1415, https://doi.org/10.1016/j.jhep.2016.02.004.
[58] M.K. Piya, P.G. McTernan, S. Kumar, Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin, J. Endocrinol. 216 (2013), https://doi.org/10.1530/JOE-12-0498.
[59] A.B. Oyenihi, N.N. Chegou, O.O. Oguntibeju, B. Masola, Centella asiatica enhances hepatic antioxidant status and regulates hepatic inflammatory cytokines in type 2 diabetic rats, Pharm. Biol. 55 (2017) 1671–1678, https://doi.org/10.1080/13880209.2017.1318293.
[60] L.R. Pessoa, T.D.S. Rêgo, S. Asht, I. Cabral, R.S. Fortunato, M. Barreto, A.M. Correia-santos, C. Alberto, G.T. Boaventura, Serum and liver lipids distributions in streptozotocin induced diabetic rat treated with diet containing Yam (Dioscorea bulbifera) flour, Nutr. Hosp. 31 (2015) 1647–1653, https://doi.org/10.3305/nh.2015.31.4.8438.
[61] P. Mondal, S. Das, J.A. Junejo, S. Borah, K. Zaman, Evaluations of antidiabetic potential of the hydro-alcoholic extract of the stem bark of Plumeria rubra a traditionally used medicinal source in North-East India, Int. J. Green Pharm. 10 (2016) 252–260, https://doi.org/10.22377/ijgpmds.
[62] W. Hu, J.H. Yeo, Y. Jiang, S. Il Heo, M.H. Wang, The antidiabetic effects of an herbal formula composed of Alnus hirsuta, Rosa davurica, Acanthopanax senticosus and Panax schinseng in the streptozotocin-induced diabetic rats, Nutr. Res. Pract. 7 (2013) 103–108, https://doi.org/10.4162/nrp.2013.7.2.103.
[63] R. Kumar, V. Arora, V. Ram, A. Bhandari, P. Vyas, Hypoglycemic and hypolipidemic effect of Allopolyherbal formulations in streptozotocin induced diabetes mellitus in rats, Int. J. Diabetes Mellit. 3 (2015) 45–50, https://doi.org/10.1016/j.ijdm.2011.01.005.
[64] B.A. Aldahmash, D.M. El-Nagar, K.E. Ibrahim, Attenuation of hepatotoxicity and oxidative stress in diabetes STZ-induced type 1 by biotin in Swiss albino mice, Saudi J. Biol. Sci. 23 (2016) 311–317, https://doi.org/10.1016/j.sjbs.2015.09.027.
[65] A. Arya, M.M. Jamil Al-Obaidi, R.B. Karim, H. Taha, A.K. Khan, N. Shahid, A.S. Sayem, C.Y. Looi, M.R. Mustafa, M.A. Mohd, H.M. Ali, Extract of Woodfordia fruticosa flowers ameliorates hyperglycemia, oxidative stress and improves β-cell function in streptozotocin-nicotinamide induced diabetic rats, J. Ethnopharmacol. 175 (2015) 229–240, https://doi.org/10.1016/j.jep.2015.08.057.
[66] A.H. Amin, M.A. El-missiry, A.I. Othman, Melatonin ameliorates metabolic risk factors, modulates apoptotic proteins, and protects the rat heart against diabetes-induced apoptosis, Eur. J. Pharmacol. 747 (2015) 166–173, https://doi.org/10.1016/j.ejphar.2014.12.002.
[67] A. Arya, M.M.J. Al-obaidi, N. Shahid, M. Ibrahim, B. Noordin, C. Yeng, W. Fen, S. Lay, M. Rais, Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of STZ-induced diabetic rats : a mechanistic study, Food Chem. Toxicol. 71 (2014) 183–196, https://doi.org/10.1016/j.fct.2014.06.010.